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Phospholipid Fatty Acid Analysis as Part of the Yucca Mountain Project Final Report

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Abstract: In support of the Yucca Mountain subsurface microbial characterization project phospholipid fatty acid (PLFA) analyses for viable microbial biomass, community composition and nutritional status were performed. Results showed a positive correlation between a decrease in viable biomass and increase in depth with the lowest biomass values being obtained from the Topopah Spring geologic horizon. A plot of the ratio of non-viable (diglyceride fatty acids) to viable (PLFA) cells also showed the lowest values to derive from the Topopah Spring horizon. Estimations of microbial community composition, made from the patterns of PLFA recovered from the sediment samples, revealed similarities between samples collected within the same geologic horizons: Tiva Canyon, Pre-Pah Canyon and Topopah Spring. Results indicated the presence of mixed communities composed of gram positive, gram negative, actinomycete and obligate anaerobic bacteria. Culturable organisms, recovered from similar sediments, were representative of the same bacterial classifications although gram positive bacterial isolates typically outnumbered gram negative isolates. Within the gram negative bacterial community, corroborative indicators of physiological stress were apparent in the Topopah Spring horizon.

Introduction: One of the goals of the research being performed at the Exploratory Study Facility (ESF) at Yucca Mountain, NV was the characterization of the *in situ* microbiota surrounding the proposed site of a high level nuclear waste repository. The presence, composition and extent of bacterial colonization of the volcanic tuffs in this area were to be measured. This characterization was necessary to address the potential for bacterial transport of radioactive materials away from the repository site and for microbially facilitated corrosion of the repository and surrounding structures.

Although classical microbiological techniques provide insight into the types and numbers of culturable organisms present in a given sample, they do not address the vast majority of the microbial community (>90%) which resides as non-culturable¹. The analyses of ester-linked phospholipid fatty acids (PLFA) were utilized to provide a quantitative measure of the *in situ* viable microbial community. The assay does not require that any organisms be cultured since it relies on organic solvents for the quantitative recovery of cellular components. Since phospholipids are turned over rapidly upon cell lysis², the recovery of intact phospholipid moieties provides a measure of the viable cells in an environmental sample. Phospholipid fatty acids differ sufficiently in molecular structure to allow for the identification of different genera and species³. Specific ratios of PLFA have been shown to reflect the physiological status of certain

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members of the extant microbiota⁴. Based on the above traits, the PLFA analysis is the appropriate assay for the characterization of the microbiota in the subsurface sediments surrounding the proposed high level nuclear waste repository.

Methods: Sediment samples of approximately 100 gram weights were received via overnight mail shipped on dry ice. The sediments were thawed and homogenized in a mill prior to extraction in a Bligh and Dyer (chloroform:methanol) solvent system modified to contain a phosphate buffer⁵. After an extraction period of approximately 3 hrs. additional aliquots of chloroform and water were added resulting in the separation of the organic lipid containing phase from the aqueous phase⁵. The organic phase was further separated on a column of silicic acid into neutral-, glyco- and polar-lipids⁶. The polar lipid fraction was then subjected to a mild alkaline methanolysis resulting in the recovery of polar-lipid (phospholipid) fatty acid methyl esters (PLFA)⁶. The PLFA were then further separated, quantified and identified by capillary gas chromatography coupled to a mass selective detector⁷.

Diglyceride fatty acids were recovered from the glyco-lipid fraction after isolation on a thin layer chromatography plate⁸. These fatty acids were then derivatized and identified in the same manner as the PLFA. The whole cell assay entailed an acid hydrolysis of a portion the organic phase⁹ followed by a strong acid methanolysis⁹ with subsequent identification by capillary gas chromatography with mass selective detection⁷.

Bacterial isolate identifications were made using the MIDI system developed by Microbial ID, Inc., Newark, DE.

The multivariate statistical analyses were performed on arcsin transformed mole percentages using the software package Einsight by Info-Metrix, Seattle, WA.

Results and Discussion (biomass): PLFA and whole cell results are provided in appendices A and B, respectively. In figure 1, a plot of the biomass estimates is made for both of these lipid fractions. The figure illustrates an average over a particular sample set of only those values above a background level of 0.60 pmole gram⁻¹ for the PLFA and 7.00 pmole gram⁻¹ for the whole cell analysis, see appendix C. A PLFA background of 0.60 pmole gram⁻¹ is roughly equivalent to 1.5×10^4 cells gram⁻¹ assuming 1 pmole of PLFA is equivalent to 2.5×10^4 cells¹⁰.

A general decrease in viable biomass occurred moving from the Tiva Canyon to the Topopah Spring horizons. This decrease in biomass was also positively correlated with depth. The lower biomasses occurred at the greater depths. Of the three geologic horizons represented in the figure, the Topopah Spring horizon showed, on average, the lowest viable biomass and the Tiva Canyon horizon the highest. In all of the sediments analyzed, viable biomass estimates were low (compared to a surface soil which typically shows 10^9 cells gram⁻¹) with the highest level of PLFA occurring in sample #3951c at 11.20 pmole gram⁻¹ or 2.8×10^5 cells gram⁻¹.

Whole cell lipid biomass was considerably greater than that of the viable biomass. The two to three order magnitude discrepancy between the two lipid classes suggests that the whole cell assay recovered lipid material not only from microbial cells but from sedimentary material (organics) as well. In a number of the samples amide and hydroxy fatty acids were detected comprising anywhere from 1 to 73% of the whole cell profile

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(appendix B). The detection of these two lipid classes could indicate an input from deposited plant material other similar type of organic material¹¹. However, certain bacterial species have been shown to contain these types of lipids and should be considered as a potential source¹².

The *Sphingomonas* species contains alpha-hydroxy fatty acids in the inner cell membrane while most all gram negative bacteria contain beta-hydroxy fatty acids in the lipopolysaccharide making up their outer membranes. The *Sphingomonas* species also contain amide fatty acids as do some gram positive genera¹². Monounsaturated fatty acids were the most abundant of the typical membrane lipids detected and were found to be primarily in the glyco-lipid fraction off of the silicic acid column. Certain gram positive bacteria, such as *Bacillus*, *Micrococcus*, or *Streptococcaceae*, contain a high percentage of glyco-lipid fatty acids. Since a number of gram positive and some gram negative bacteria were isolated from the sediments collected at the ESF, it is likely that bacteria contributed, at least to some extent, to the whole cell biomass estimate.

In figure 1, whole cell (or total) biomass was observed to be greatest in the pre-Pah canyon samples. These samples showed approximately a 50-50 split between typical membrane fatty acids and amide fatty acids. On average, the Topopah Spring sediments showed a similar whole cell biomass to that observed in the Tiva Canyon sediments. The nature of the whole cell assay precludes any ability to separate those acids which are likely of a microbial origin from those which are not. As a result, it is not feasible to attempt to estimate the microbial biomass versus that of another organic source.

The isolation of the PLFA and the diglyceride fatty acid (DGFA) moieties allows for the estimation of the number of non-viable to viable microbial cells present in the sediment samples. Recently lysed cells are exposed to phospholipases which cleave the phosphate headgroup from the glycerol backbone resulting in a moiety called a diglyceride. The fatty acids of the diglyceride are the same as those when the molecule was a phospholipid. The plot in figure 2 shows that the ratio of DGFA/PLFA was highest in the pre-Pah Canyon horizon, which also showed the greatest whole cell (or total) biomass. The number of non-viable cells in relation to viable cells decreased as the ESF traversed the Topopah Spring horizon. This result suggests that near the site of the proposed repository those microbial cells that are present are primarily viable living cells, whereas through the Tiva and pre-Pah Canyon horizons the number of recently lysed cells is equal to or greater than the number of viable cells present. These results can be interpreted as indicating the Topopah Spring organisms are considerably less active than those in the other two horizons and that there is little turnover within the microbial community.

(community composition) The patterns of PLFA detected in the sediment samples showed similarities which were attributed to the geologic horizon from which they were recovered (figure 3). These relationships could also be described by the depth from which the sediments were collected and by the viable biomass present, both of which are related to the geologic horizons. In figure 4, the relative percentages of specific groups of bacteria and micro-eukaryotes were highlighted. Interestingly, gram positive specific PLFA represented a much smaller percentage of the total PLFA than did the gram negative biomarkers. This is in contrast to the culturable results which consistently (sample to sample) showed a greater number of gram positive isolates than gram negative,

as identified by the MIDI system. The contribution of glyco-lipids to any gram positive bacterial presence is overlooked in this plot and the percentage, based on the two specific PLFA biomarkers in figure 4, may be an underestimation. On average, the gram positive bacterial percentage decreases along the ESF from the Tiva Canyon to the Topopah Spring horizons whereas the gram negative bacterial biomarker percentage increases.

The micro-eukaryote biomarker, 18:2w6, was detected throughout the ESF. This PLFA is prominent in fungal cell membranes as well as some protozoans. The sulfate reducing bacterial biomarkers were found to be greatest in percentage in the pre-Pah Canyon samples. Since these bacteria have been associated with microbially facilitated corrosion, they are of concern as a potential contributor to structural deterioration. The only Topopah Spring sediment to show the presence of these biomarkers was sample #7889 which was collected at the beginning of the horizon.

As previously mentioned, the majority of identifications of bacterial isolates cultured from the Yucca Mountain sediments were identified by the MIDI system as gram positive. Table 1 lists the number of isolates analyzed, from which sample they were cultured and their identification as either a gram positive or a gram negative genus. Where identifications were not made, a matched pairs hierarchical cluster analysis of normalized area percentages was used to relate the unknown isolates to identified ones.

Results of the MIDI analysis also indicated the possible recovery of isolates from the *Sphingomonas* genus. Of the 244 isolates analyzed, 11% showed a fatty acid pattern not unlike a sphingomonad by showing the characteristic presence of alpha-hydroxy fatty acids in the absence of beta-hydroxy acids. This genus of bacteria is very important in the context of bioremediation technologies since they have been demonstrated to contain the capacity to degrade a large number of xenobiotics. Their presence in a sediment or geologic horizon could be beneficial in future bioremediation efforts.

Table 1. The number (and percentage) of isolates cultured from the Yucca Mtn. sediments which were identified as either a gram(+) or a gram(-) genus by the MIDI system.

sample #	gram(+)	gram(-)	total
#3951	17 (71%)	7 (29%)	24
#7891	30 (61%)	19 (39%)	49
#7888	3 (50%)	3 (50%)	6
#7889	7 (88%)	1 (12%)	8
#9299	24 (92%)	2 (8%)	26
#7893	10 (91%)	1 (9%)	11
total # of isolates	91 (73%)	33 (27%)	124

(physiological status) Within the gram negative classification of bacteria, specific ratios of PLFA have been identified which are related to a cells physiological status⁴. In figure 5, a plot of these two ratios indicates that the gram negative populations in the Topopah Spring sediments exhibited a higher degree of physiological stress than in the other two horizons. The ratio of the cyclopropyl fatty acid to the monounsaturated precursor (cy17:0/16:1w7c) has been shown to increase as cultures enter a stationary

phase of growth. The observed increase in this ratio in the Topopah Spring sediments correlates with the lower observed viable biomass and decreased ratio of DGFA/PLFA (assuming this later ratio is indicating a lack of microbial activity). The ratio of *trans/cis* isomers (16:1w7t/16:1w7c) has been shown to increase in starved cultures as well as cultures exposed to toxicants. The Topopah Spring sediments were the only sediments analyzed which showed the presence of *trans* fatty acids. The observation of increased ratios for both of these stress markers suggests the extant (gram negative) microbiota in the Topopah Spring sediments were undergoing some form(s) of environmental stress.

(controls) Two of the samples were identified as containing very unique PLFA patterns characteristic of a typical *Pseudomonas* sp. and PLFA concentrations two orders of magnitude above the other sediment samples (figure 2). These two samples proved to be spiked controls. A number of other samples were autoclaved prior to shipment. Although the autoclave process disrupts cell membranes and renders the cells non-viable, the heat also denatures enzymes. As a result, phospholipids can remain intact in sediments after autoclaving. In order to purify sediments of lipid components, combustion at 400°C is required.

Conclusion: The detection of PLFA above background levels indicated the presence of viable bacteria within the volcanic tuffs along the ESF. Although the estimate of viable cell numbers was lowest among the Topopah spring sediments, detectable levels of 10^4 to 10^5 cells per gram⁻¹ were observed. The composition of the extant viable microbial communities was diverse showing characteristic PLFA of a number of different genera. PLFA results indicated that gram negative populations comprised a greater percentage of the community than did the gram positive bacteria even though more gram positive organisms were cultured from the sediments. Indications for a gram positive presence were also found in the whole cell analysis suggesting that the PLFA estimate may be underestimating this component of the community. The whole cell and the MIDI analysis also provided evidence for what is believed to be *Sphingomonas* sp. which have favorable bioremediation implications. The increased percentage of gram negative bacterial biomarkers was associated with an increase in the magnitude of physiological stress indicators. This result indicated that the gram negative community was undergoing some form of environmental stress within the Topopah Spring geologic horizon that was not apparent in the other horizons. The above described results suggests that although bacterial colonization (if not persistence) of the sediments surrounding the proposed repository site has occurred, the scope of the colonization appears to be small (on the order of 10^4 cells gram⁻¹) and under less than ideal conditions (indications of physiological stress).

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Figure 1. Biomass estimates based on the concentrations of polar lipid fatty acids (viable biomass) and whole cell lipids (total biomass) detected in Yucca Mtn. sediments.

Figure 2. Estimate of the ratio of non-viable to viable (DGFA/PLFA) microbial biomass in Yucca Mtn sediments. Non-viable cells are estimated from the diglyceride fatty acid concentration and viable cells from the polar lipid fatty acid concentration.

Figure 3. Hierarchical cluster analysis of the arcsin transformed mole percentages of PLFA recovered from Yucca Mtn. sediments. Samples which were autoclaved or that showed a total PLFA biomass below 0.60 pmole/g were not included.

Figure 4. Estimates of microbial community composition in Yucca Mtn. sediments.

gram(+) = i15:0 + a15:0, gram(-) = 16:1w7c + 18:1w7c, SRB = 10me16:0 + i17:1, eukaryotes = 18:2w6

Figure 5. Estimations of the gram (-) microbial community metabolic status.

Appendix A - polar lipid fatty acid profile.

	(pmole/g)		(mole %)									(ratio)				
	DGFA	PLFA	gram(+)	gram(-)	SRB	nsat	midbrsat	terbrsat	mono	brmono	poly	DGFA/PLFA	16:1w7U7c	18:1w7U7c	cy17:0/7c	cy19:0/7c
#3951a	0.58	1.89	1.26	2.05	1.41	85.10	1.62	2.37	9.35	0.28	1.29	0.31	-	-	0.87	0.11
#3951c	6.00	11.20	0.22	0.87	0.11	97.69	0.27	0.53	1.35	-	0.16	0.54	-	-	-	-
#3951d	0.49	1.32	1.32	2.95	0.85	75.65	1.81	2.82	18.39	0.14	1.19	0.37	-	-	0.14	-
#3951e	0.79	10.12	0.32	0.40	0.26	96.26	0.46	0.85	1.70	0.06	0.68	0.08	-	-	0.25	0.32
#3951f	0.49	1.60	1.00	2.53	0.49	80.49	0.85	2.81	14.66	-	1.19	0.31	-	-	-	-
#7891a	1.09	1.31	1.96	5.21	-	69.25	0.25	3.84	23.72	-	2.94	0.83	-	-	0.10	-
#7891b	1.53	0.89	1.11	3.55	-	79.16	0.26	2.56	16.76	-	1.28	1.73	-	-	-	-
#7891c	2.11	5.08	0.13	0.91	0.09	94.83	0.14	0.81	4.06	-	0.16	0.42	-	-	0.85	-
#7891d	0.51	0.56	3.67	4.27	-	73.47	0.34	6.59	18.23	-	1.37	0.90	-	-	0.33	-
#7891e	0.45	0.65	1.70	4.58	-	69.94	0.51	4.24	24.32	-	0.99	0.69	-	-	1.19	-
#7888a	0.54	2.98	0.89	9.78	1.94	70.15	1.94	2.09	25.60	-	0.22	0.18	-	-	-	-
#7888b	0.11	2.53	0.65	8.76	1.25	61.61	1.25	2.14	32.82	-	2.19	0.04	-	-	0.30	0.03
#7888c	0.16	3.86	0.65	6.77	1.31	59.85	1.31	2.56	33.75	-	2.53	0.04	-	-	0.24	0.05
#7888d	1.54	1.04	0.57	7.39	2.70	60.79	2.70	2.85	30.61	-	3.05	1.48	-	-	-	-
#7888e	4.46	0.89	0.42	8.11	1.11	64.61	1.11	2.99	28.98	-	2.31	5.01	-	-	0.24	0.08
#7888f	5.33	0.66	0.89	8.18	3.16	66.72	3.16	3.47	25.06	-	1.60	8.08	-	-	-	-
#7889a	0.17	1.55	1.96	6.86	-	60.56	-	5.43	31.26	-	2.76	0.11	-	-	-	-
#7889b	2.54	1.00	0.33	5.99	0.59	73.34	0.59	1.80	22.52	-	1.75	2.54	-	-	0.32	-
#7889c	1.84	0.87	0.84	5.87	0.93	73.43	0.93	2.65	21.33	-	1.67	2.11	-	-	-	-
#7889d	3.62	0.32	0.71	5.87	1.33	75.20	1.33	1.72	20.29	-	1.45	11.31	-	-	0.35	-
#7889e	0.15	0.37	-	6.58	0.72	72.89	0.72	0.97	23.95	-	1.47	0.41	-	-	-	-
#9299a	1.71	0.37	-	10.32	-	60.32	-	0.46	37.57	-	1.65	4.62	0.18	-	0.66	-
#9299b	0.2	0.38	-	7.86	-	63.14	-	-	36.86	-	-	0.53	2.24	-	2.13	-
#9299c	0.15	0.42	-	10.16	-	73.68	-	-	25.09	-	1.22	0.36	0.24	-	0.61	-
#9299d	0.13	0.62	-	9.03	-	65.10	-	-	33.78	-	1.12	0.21	0.16	-	0.77	-
#9299e	0.13	1.27	-	3.60	-	88.79	-	-	10.77	-	0.44	0.10	0.27	-	0.75	-
#7893a	0.08	0.98	0.32	10.27	-	65.38	-	0.64	32.42	-	1.57	0.08	0.26	-	0.71	-
#7893b	0.21	2.09	0.26	21.04	-	54.83	-	0.72	43.74	-	0.72	0.10	0.20	-	0.82	0.04
#7893c	0.17	0.46	0.62	13.29	-	61.45	-	0.62	37.24	-	0.69	0.37	0.25	-	0.72	-
#7893d	0.4	0.36	-	15.09	-	62.48	-	-	36.72	-	0.80	1.11	0.26	-	0.80	0.16
#7893e	0.34	185.89	0.09	37.37	-	39.08	-	0.40	60.50	-	0.02	0.00	0.25	0.03	0.62	0.02
#9298a	0.14	0.39	-	10.26	-	62.94	-	1.86	34.16	-	1.04	0.36	0.27	-	0.75	0.56
#9298b	0.21	0.44	0.56	11.12	-	67.42	-	1.56	29.93	-	1.09	0.48	0.20	-	0.53	0.27
#9298c	0.19	0.43	0.46	13.40	-	63.86	-	0.46	34.11	-	1.57	0.44	0.11	-	0.43	0.14
#9298d	0.09	223.88	-	50.21	-	29.45	-	0.22	70.31	-	0.01	0.00	0.21	0.02	0.34	0.01
#9298e	0.10	29.33	0.03	42.89	-	40.85	-	0.47	58.65	-	0.04	0.00	0.19	0.02	0.27	0.01
#9260a	0.09	1.09	-	7.08	-	84.77	-	-	14.47	-	0.76	0.08	0.21	-	0.59	-
#9260c	0.14	0.09	-	14.54	-	66.80	-	-	32.32	-	0.88	1.56	0.30	-	1.16	-
#9260h	0.11	0.07	-	11.32	-	71.87	-	-	26.32	-	1.81	1.57	-	-	0.63	-
#9260m	0.28	0.14	1.38	8.69	-	72.74	-	1.38	22.72	-	3.16	2.00	0.34	-	0.27	-
#9260q	0.24	0.09	-	6.81	-	77.17	-	-	21.43	-	1.40	2.67	-	-	1.47	-
#9260z	0.35	0.07	1.94	8.21	-	69.85	-	1.94	26.50	-	1.71	5.00	-	-	1.80	-

DGFA = diglyceride fatty acid methyl esters

PLFA = polar lipid fatty acid methyl esters

gram(+) = sum of 11:5:0 and a15:0

gram(-) = sum of 16:1w7c and 18:1w7c

SRB = sulfate reducing bacteria, sum of 10me16:0 and 11:7:1

nsat = normal saturates

midbrsat = mid-chain branched saturates

terbrsat = terminally branched saturates

mono = monounsaturates

brmono = branched monounsaturates

poly = polyunsaturates

DGFA/PLFA = diglyceride fatty acid pmole/g to polar lipid fatty acid pmole/g

16:1w7U7c = 16:1w7t to 16:1w7c

18:1w7U7c = 18:1w7t to 18:1w7t

cy17:0/7c = cy17:0 to 16:1w7c

cy19:0/7c = cy19:0 to 18:1w7c

Appendix B - whole cell lipid profile.

	(pmole/g)	(mole %)														
	biomass	nsat	terbrsat	mono	brmono	poly	TotFAME	DMA	FATMS	dioic	alc	amide	OHFAME	oxoFA	unk	squalene
#3951a	2.47	3.38	-	83.98	-	-	87.37	-	-	-	-	-	12.63	-	-	-
#3951c	10.10	16.63	0.58	39.65	-	0.55	57.40	-	0.75	-	-	-	41.84	-	-	-
#3951d	135.58	4.30	0.93	69.10	-	1.02	75.35	0.08	0.20	-	0.07	16.95	6.02	0.63	0.71	-
#3951e	57.74	3.12	0.02	14.49	-	0.09	17.72	-	0.04	-	-	73.53	7.39	0.04	0.29	0.98
#3951f	318.72	3.85	1.64	73.85	0.42	1.75	81.51	-	0.43	-	0.43	9.73	4.29	1.80	1.82	-
#7891a	662.19	3.21	1.21	68.72	0.14	1.53	74.82	-	0.30	-	0.20	20.42	2.46	0.85	0.95	-
#7891b	677.86	2.17	0.85	45.89	0.08	1.08	50.07	-	0.37	-	0.08	44.12	3.39	1.25	0.74	-
#7891c	1180.93	1.98	0.55	30.14	1.23	0.74	34.63	-	0.12	-	0.12	61.60	2.73	0.22	0.31	0.27
#7891d	169.42	2.96	1.12	56.95	0.10	1.47	62.60	-	0.69	-	0.32	28.85	2.96	2.95	1.49	0.13
#7891e	265.84	1.82	0.65	29.87	0.15	0.89	33.18	-	0.66	-	0.34	58.85	2.50	2.40	1.57	0.49
#7888a	1244.66	6.59	1.65	24.55	2.03	2.09	36.90	0.11	2.88	-	1.54	48.97	6.47	1.26	1.79	0.07
#7888b	1405.69	3.84	1.04	43.03	-	1.33	49.24	-	1.41	-	0.38	41.71	4.67	1.06	1.46	0.07
#7888c	1074.87	3.97	0.86	35.51	-	1.21	41.56	0.07	0.72	-	0.26	49.57	4.95	1.44	1.43	-
#7888d	1363.53	2.36	0.87	32.59	-	1.10	36.91	-	1.10	-	0.27	55.35	4.04	1.03	1.16	0.13
#7888e	872.40	4.13	1.31	62.84	0.20	1.85	70.33	-	0.63	-	0.38	20.64	3.72	1.59	2.53	0.19
#7888f	818.41	1.60	0.60	29.52	-	0.57	32.28	0.09	0.69	-	0.27	61.19	3.47	1.19	0.83	-
#7889a	1171.53	2.92	1.16	54.39	-	1.15	59.62	-	0.69	-	0.36	32.81	3.48	1.29	1.69	0.05
#7889b	177.85	15.72	3.00	31.33	-	1.45	51.50	-	2.12	-	3.35	19.83	16.96	3.24	2.44	0.55
#7889c	477.24	2.69	1.15	53.73	-	1.48	59.05	-	0.56	-	0.24	30.57	3.81	2.35	3.42	-
#7889d	569.23	2.91	1.02	55.83	-	1.31	61.06	-	0.42	-	0.41	28.94	4.65	1.52	3.00	-
#7889e	594.66	3.62	1.19	55.93	-	1.48	62.21	-	0.59	-	0.38	27.44	4.10	1.89	3.38	-
#9299a	837.09	6.57	1.67	69.57	-	1.84	79.66	0.24	2.14	0.11	0.77	5.11	11.37	-	0.60	-
#9299b	673.61	7.41	1.79	68.61	-	2.15	79.97	0.78	1.31	0.21	0.66	5.27	11.12	-	0.68	-
#9299c	857.11	6.83	1.78	73.19	-	2.48	84.28	0.27	1.59	0.14	0.34	2.51	10.15	-	0.72	-
#9299d	56.18	10.23	-	53.31	-	-	63.53	0.85	2.59	-	-	-	33.03	-	-	-
#9299e	173.11	8.00	0.78	57.43	-	0.99	67.20	0.06	1.07	-	0.49	0.92	29.29	-	0.96	-
#7893a	1013.13	7.30	2.08	63.89	-	1.71	74.97	0.30	1.49	0.51	0.91	9.88	10.31	-	1.63	-
#7893b	1041.91	7.65	2.10	64.78	-	0.48	75.02	0.99	2.10	0.67	1.03	8.14	9.28	-	2.76	-
#7893c	230.78	6.06	1.50	67.71	-	1.08	76.35	0.41	1.45	0.16	-	1.80	17.95	-	1.87	-
#7893d	308.08	5.46	1.41	66.36	-	1.56	74.78	0.53	7.78	0.10	1.04	-	14.00	-	1.76	-
#7893e	1006.98	21.09	0.91	64.32	-	0.62	86.94	0.07	1.74	0.42	0.54	2.51	7.63	-	0.15	-
#9298a	410.20	5.76	1.62	65.14	-	1.06	73.57	0.51	3.59	0.29	1.75	2.40	16.28	-	1.61	-
#9298b	250.32	6.38	1.42	60.73	-	1.67	70.21	0.12	5.42	-	0.56	2.77	18.61	-	2.31	-
#9298c	368.70	4.62	0.79	76.41	-	1.31	83.14	-	0.35	-	0.21	3.12	10.56	-	2.62	-
#9298d	1309.05	25.50	0.77	42.38	0.23	0.80	69.69	0.04	18.60	0.37	1.15	2.13	8.02	-	-	-
#9298e	658.55	6.46	1.31	69.10	-	1.56	78.43	0.22	4.73	0.37	0.55	2.58	12.49	-	0.64	-
#9260a	405.21	6.20	1.39	58.75	-	1.98	68.32	0.77	19.85	0.30	0.64	1.86	7.48	-	0.78	-
#9260c	290.21	6.53	1.57	57.59	-	1.52	67.21	0.71	17.49	2.19	0.91	2.25	8.72	-	0.51	-
#9260h	167.96	6.37	1.15	54.62	-	1.15	63.28	0.89	23.09	1.66	0.56	-	7.42	-	3.10	-
#9260m	379.10	6.56	1.80	59.90	-	1.53	69.79	1.30	20.97	0.34	0.49	0.91	5.14	-	1.06	-
#9260q	266.23	7.16	1.36	60.19	-	1.61	70.32	0.52	19.89	1.32	0.57	0.76	5.11	0.00	1.52	-
#9260z	220.89	6.42	1.24	60.80	-	1.62	70.08	0.69	20.59	1.32	0.37	-	5.93	-	1.02	-

biomass = total pmole/gram
 nsat = normal saturates
 terbrsat = terminally branched saturates
 mono = monounsaturates
 brmono = branched monounsaturates

poly = polyunsaturates
 TotFAME = total fatty acid methyl esters
 DMA = dimethyl acetals
 FATMS = trimethyl sited fatty acids
 dioic = di-carboxylic acids

alc = trimethyl sited alcohols
 amide = amide fatty acids
 OHFAME = trimethyl sited hydroxy fatty acids
 oxoFA = trimethyl sited oxo fatty acids
 unk = unknown compounds

Appendix C - procedural blank summary

whole cell lipid profiles

pmol/g	run-1	run-2	run-3	run-4	average	std.dev
Total	0.12	6.97	19.94	3.57	7.65	7.50
FAME	0.06	3.95	3.90	1.14	2.26	1.71
TMSFA	-	-	7.84	0.87	2.18	3.29
OHFAME	0.06	-	8.20	1.56	2.46	3.38
unk	-	3.02	-	-	0.76	1.31

polar lipid fatty acid profiles

pmol/g	run-1	run-2	run-3	run-4	average	std.dev
Total	0.01	0.13	2.51	0.04	0.67	1.06
nsat	0.01	0.11	1.21	0.03	0.34	0.50
mono	-	0.02	1.30	0.01	0.33	0.56

Total diglyceride fatty acids

pmol/g	run-1	run-2	run-3	average	std.dev
Total	-	0.14	-	0.05	0.07